

IN THE CLAIMS:

Please cancel claims 174, 178, 182, and 186, without prejudice or disclaimer. Please amend the claims pursuant to 37 C.F.R. 1.121 as follows (see the accompanying "marked up" version pursuant to 1.121):

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D' 156. (Twice amended) A cytochrome P450 oxygenase variant having a catalytic activity at least two times the catalytic activity of wild-type cytochrome P450<sub>cam</sub> oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

157. (Twice amended) A cytochrome P450 oxygenase variant having a catalytic activity at least about ten times the catalytic activity of wild-type cytochrome P450<sub>cam</sub> oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

158. (Twice amended) A cytochrome P450 oxygenase variant having a stability at least two times the stability of wild-type cytochrome P450<sub>cam</sub>

oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

D<sup>1</sup>  
cont.

159. (Twice amended) A cytochrome P450 oxygenase variant having a stability at least about ten times the stability of wild-type cytochrome P450<sub>cam</sub> oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

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D<sup>2</sup>

161. (Twice amended) An oxygenase variant evolved from a wild-type oxygenase enzyme, and having a catalytic activity at least ten times the catalytic activity of the wild-type oxygenase enzyme in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, which oxygenase variant was identified by a method comprising the steps of:

(a) contacting a test enzyme variant with an oxygenase substrate and the oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme;

(b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product;

(c) detecting the detectable composition; and

*D<sup>2</sup>  
cont.*

(d) selecting any test enzyme having at least 10 times the catalytic activity of the wild-type oxygenase enzyme in the presence of the oxygen donor, at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to one of amino acids 242, 280, and 331 of SEQ ID NO:2.

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*D<sup>3</sup>*

164. (Amended) An oxygenase variant evolved from a wild-type oxygenase enzyme, and having a stability at least ten times the stability of the wild-type oxygenase enzyme in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, which oxygenase variant was identified by a method comprising the steps of:

(a) contacting a test enzyme variant with an oxygenase substrate and the oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme;

(b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product;

(c) detecting the detectable composition; and

D3  
cont,

(d) selecting any test enzyme having at least 10 times the stability of the wild-type oxygenase enzyme, at least 90% sequence identity to SEQ ID NO:2, and a mutation in at least one position corresponding to at least one of amino acids 242, 280, and 331 of SEQ ID NO:2.

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D4

167. (Amended) A functional cytochrome P450 oxygenase variant identified by a method comprising the steps of:

(a) contacting a test cytochrome P450 oxygenase variant with an oxygenase substrate and an oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme;

(b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product;

(c) detecting the detectable composition; and

(d) selecting any test enzyme having a mutation at a position corresponding to at least one of amino acid 331, 280, and 242 of cytochrome P450<sub>cam</sub> from *P. putida* (SEQ ID NO:2) and at least 90% sequence identity to SEQ ID NO:2.

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D<sup>5</sup> 175. (Amended) The cytochrome P450 variant of claim 156,  
comprising at least one mutation selected from lysine at amino acid 331, leucine at  
amino acid 280, and phenylalanine at amino acid 242.

D<sup>6</sup> 179. (Amended) The cytochrome P450 variant of claim 157,  
comprising at least one mutation selected from lysine at amino acid 331, leucine at  
amino acid 280, and phenylalanine at amino acid 242.

D<sup>7</sup> 183. (Amended) The cytochrome P450 variant of claim 158,  
comprising at least one mutation selected from lysine at amino acid 331, leucine at  
amino acid 280, and phenylalanine at amino acid 242.

D<sup>8</sup> 187. (Amended) The cytochrome P450 variant of claim 159,  
comprising at least one mutation selected from lysine at amino acid 331, leucine at  
amino acid 280, and phenylalanine at amino acid 242.